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Preparation and immunogenicity of vaccine Ac NFU_1 (S^-) MRC towards the prevention of herpes genitalis

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SUMMARY A subunit antigenoid vaccine, Ac NFU_1 (S^-) MRC, was used to prevent primary herpes genitalis in 60 subjects considered to be at risk of this infection. There was no evidence of serious local or general side effects.

Neutralising antibody responses were detected in 59% and 90% of subjects receiving the low and high doses of vaccine respectively; immunoprecipitating antibody was detected at a lower frequency, namely in 23% and 43% of subjects receiving the low and high doses respectively. After a mean follow-up period of 18 months none of the vaccinated subjects contracted herpes genitalis after completing the vaccination course.

Introduction

Herpes genitalis is an acutely painful sexually transmitted disease (STD) whose prevalence is rapidly increasing throughout the United Kingdom[1]; moreover, as patients will remain infectious during and to some extent between clinical recurrences the prevalence of this disease, unlike other STDs, will, if left unchecked, increase. Similar anxieties arise with the complications of this infection; for example, the management of the pregnant patient with herpes genitalis is now a regular problem in our maternity centre. Finally, the large body of molecular and both retrospective and prospective epidemiological evidence associating this infection with the later development of preinvasive and invasive cervical carcinoma[2-8] emphasises the urgency of instituting measures for preventing this infection not only for the individual patient but for the community as a whole.

The feasibility of immunisation against herpes simplex infection was first shown in rabbits by Lipchutz[9] and more recently against primary type 2 herpes virus infection using subunit or inactivated type 1 or type 2 vaccine preparations.[10-15] Of particular importance are a number of studies reporting varying degrees of protection against latent type 2 ganglionic infection in mice immunised with live or inactivated type 2 virus vaccines[16-18] or type 1 vaccine preparations.[19-21]

It is curious therefore how little effort has been directed towards the prevention of primary herpes simplex infections by vaccination of as yet uninfected subjects. The relative ease of autoreinoculation of human subjects with live herpes virus[22-24] has been discouraging as was an unsuccessful attempt to immunise 7-11-month-old orphaned children against primary type 1 herpes infections.[25] There must, however, be reservations about the immunogenic potency of the vaccine preparations in this study and the criteria of assessment of vaccine efficacy.

The present study reports the preparation, immunological response, and clinical outcome after immunisation with vaccine Ac NFU_1 (S^-) MRC of 34 seronegative and 26 seropositive subjects at risk of genital herpes virus infection.

Subjects and methods

The regular sexual consorts of 60 patients with recurrent genital herpes (index cases) were offered vaccination. The frequency, duration, severity, and

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location of herpetic disease in the index cases, the frequency of sexual relations, mode of contraception, and duration of the consortship were recorded. Parallel information was obtained from a control group of consorts at risk, namely the regular sexual partners of 20 consecutive patients who attended our clinic with primary or recurrent herpes genitalis before our preventative vaccination programme was begun. There was no history of herpes genitalis in either the vaccinated or unvaccinated subjects.

The vaccinated group of consorts (29 men and 31 women) had a mean age of 23·1 years; the unvaccinated subjects (12 men and eight women) had a mean age of 24·2 years. The difference in the socioeconomic status of the vaccinated and unvaccinated consorts was 16%, 57%, and 27% in the high (I and II), middle (III), and low (IV and V) socioeconomic groups respectively compared with 45%, 40%, and 15%. The vaccinated subjects tended therefore toward a lower socioeconomic status.

The sociosexual inter-relationships between the vaccinated and unvaccinated groups seemed comparable although in a study of this size it was not feasible to compare precisely certain sex-related variables—for example, frequency, duration, and mode of sexual relations in a given index-consort coupling. In both vaccinated and unvaccinated groups about 30% of consorts were the marital partners and the remaining 70% were the regular sexual partners of the index case; similarly the risk of exposure to herpes infection from the index case was comparable where the mean frequency of recurrences was 6·6 per year in the vaccinated and 6·0 per year in the unvaccinated consort group. There were no detectable differences in contraceptive practice or precautionary measures during herpetic recurrences between the vaccinated and unvaccinated subjects.

The subjects were followed-up for a mean of 18 months (range 4-26 months) with monthly assessment during the first three months, a further assessment at six months, and assessment at six-monthly intervals thereafter. A history of clinical features of herpes genitalis was sought at each visit; subjects were also asked to record any signs or symptoms which might be related to herpes genitalis and to report them to the responsible medical practitioner. As the principal aim of this study was to investigate the prevention of clinically overt herpes genitalis, vaginal material for virological examination was not taken unless there was clinical suspicion of herpes genitalis.

VACCINATION PROCEDURE

Two subcutaneous vaccinations were given at monthly intervals in the deltoid muscle. For the first 50 vaccinations, a test dose of 0·05 ml was administered; as no untoward reactions were observed, this precaution was omitted unless there were particular indications—for example, a history of allergic reactions, anaphylaxis, or penicillin hypersensitivity.

Two doses of vaccine were used. Thirty-nine consorts were immunised with the lower dose of 2×10^7 cell equivalents; as experience with the vaccine increased 21 consorts, identified as seronegative for neutralising antibody to type 1 or type 2 herpes simplex virus, were immunised with the higher dose of 10^8 cell equivalents.

Serum samples were obtained before vaccination and one month after the last vaccination.

Cells

MRC 5 cells, a human embryonic lung cell line, obtained from the National Institute for Biological Standards and Controls, London, were used for vaccine production and for the isolation, cloning, and preparation of virus stocks for vaccine production.

BHK 21, a stable line of baby hamster kidney cells,[26] were used for virus titration.

Virus strains

Type 1 strain (troisbell) was used for vaccine preparation. The strain was isolated and cloned three times in MRC 5 cells. Virus stocks were stored in serum-free medium. Type identification of virus strains was confirmed by neutralisation kinetics and Ouchterlony gel diffusion tests.[27]

Strains HFEM and strains 3345 were used respectively as prototype type 1 and type 2 strains for virus antigen preparation and for neutralisation tests.

Virus antigen

Type 1 and type 2 herpes simplex virus antigens for Ouchterlony immunodiffusion tests were prepared by high multiplicity infection of BHK 21 cells.[28]

VACCINE PREPARATION

Vaccine was prepared as previously described.[28a] MRC 5 human embryo lung cells were infected with strain troisbell of herpes simplex virus. Cell nuclei were removed by low-speed centrifugation after treatment of the cells with Nonidet NP40, which also strips important virus antigens from the envelope of the virus particles; formaldehyde was added to inactivate viral and host cell DNA and to stabilise virus antigens during subsequent preparative procedures. Virus particles, which although inactivated might contain biologically active DNA, were then removed by ultracentrifugation over 20% sucrose at 85 000 × *g* for five hours. The protein constituents of the vaccine preparation were precipitated by cold acetone and the precipitate washed in acetone, which was then removed by

evaporation. The dried pellet was stored at −70°C in a dehydrated container or for short-term storage at 4°C.

The safety, immunogenicity, and protective efficacy of vaccine preparations has been previously reported.[11-13 29 30] Before inoculation into human subjects vaccine batches were tested by subcutaneous inoculation of 0·05 ml (5 × 10^6 cell equivalents) into the dorsal skin of newborn mice.

NEUTRALISATION TESTS

Sera were tested by neutralisation kinetics at a final 1/10 dilution in PBS. K values were calculated for each serum as previously described.[4]

IMMUNODIFFUSION TESTS

Vaccine, antigen preparations, and human sera were tested in Ouchterlony immunodiffusion gels as previously described.[28]

POLYACRYLAMIDE GEL ELECTROPHORESIS

Vaccine preparations were analysed on 18 × 11 cm SDS-polyacrylamide slab gels cross-linked with NN' diallyl tartar-diamide. Procedures for fixing, staining, autoradiography, and calibration with molecular weight standards have been described.[31]

DETECTION OF POLYPEPTIDES BY REACTION WITH ANTISERUM AND IODINATED PROTEIN A

After thorough washing gels were incubated in appropriate dilutions of antiserum. Unreacted serum was removed by extensive washing and bound immunoglobulin detected by incubation with iodinated protein A. Radioactive proteins were detected by autoradiography.

Results

EFFICACY OF VACCINE

Polypeptide composition

The polypeptide composition at the three stages of preparation procedure is shown in the figure. The vaccine polypeptide profile is virtually indistinguishable from the profile of type 1 virus infected cell extract, with easy identification of the polypeptides whose molecular weights correspond to the polypeptides of the virus particle[31]; in particular, glycosylated polypeptides in the molecular weight range 118 000-132 000 and 55 000-63 000 were identified in every vaccine preparation. These are components of the virus envelope and have been shown to stimulate both type-specific and type-common neutralising antibodies in experimental rabbits.[32 33] The antigenicity of virus polypeptides in the vaccine has been confirmed by in-situ identification on polyacrylamide gels using hyperimmune rabbit antiserum to type 1 herpes virus infected cells.

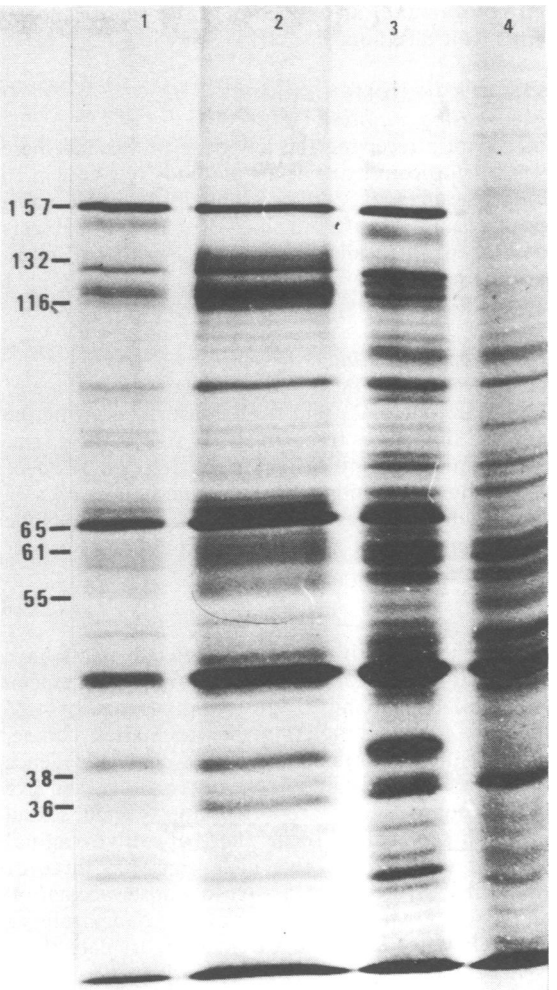

FIGURE *Polypeptide composition of vaccine at three stages of preparation by reaction with antiserum and iodinated protein A. Track 1, initial virus-infected MRC 5 cell extract after treatment with Nonidet NP40 and formaldehyde; track 2, final vaccine; track 3, virus-infected baby hamster kidney cells; and track 4, uninfected baby hamster kidney cells. (The numbers indicate apparent molecular weights × 10^{-3}.)*

Immunoprecipitating virus antigens

Vaccine preparations were routinely tested against hyperimmune type 1 antisera in Ouchterlony immunodiffusion. Under these circumstances one or two immunoprecipitins were detected; one of these immunoprecipitins was invariably band II antigen

(glycoprotein d), an antigen of major significance in virus neutralisation.[32]

STUDIES IN HUMAN SUBJECTS

Neutralising antibody

In consorts receiving the low dose of vaccine there was a significant neutralising antibody response in 10 of 13 seronegative subjects and in 13 of 26 seropositive subjects; after the high dose 19 of 21 subjects developed neutralising antibody with a mean neutralisation rate constant of 0·37 against type 1 and 0·29 against type 2 virus (table).

Immunoprecipitating antibody

One immunoprecipitin line was identified in three of 13 and in nine of 21 sera from consorts receiving the low and high doses respectively. In five of these sera, two immunoprecipitin lines were detected. One of the immunoprecipitin lines was common to all sera that reacted and was identified as anti-band II. None of the sera reacted against uninfected baby hamster kidney cell extract.

Clinical efficacy

At the time of writing, 60 vaccinated patients have been followed up for 920 patient months with a mean length of consortship of 18 months (range of 4-26 months). After completing the vaccination course, none of the consorts has developed clinical evidence of herpes genitalis; it is also noteworthy that five female subjects have reported unprotected sexual intercourse during recurrence of vulvo-vaginal herpetic disease with five male consorts, three of whom had sero-converted after low-dose vaccination and two of whom had not developed neutralising antibody after low-dose vaccination; to date there has been no clinical or serological evidence of herpes genitalis in any of these male consorts at risk. The reciprocal situation—that is unprotected exposure of vaccinated female subjects to herpes genitalis—has not as yet been reported in our study.

One consort, who had received her first vaccination one week previously, reported an episode which resembled a mild attack of herpes genitalis; there was a history of recent unprotected exposure to penile herpes from her marital partner. The patient did not attend for a further two weeks, at which time there was no clinical evidence of herpes genitalis although herpes simplex virus was isolated from the genital tract. While the features of this episode are not typical of primary herpes genitalis, and it is of course possible that herpes simplex virus was isolated after postcoital deposition and extracellular survival of virus in the genital tract, we feel obliged to record the episode as primary herpes genitalis occurring one week after first vaccination.

In 20 unvaccinated consorts, eight contracted primary herpes genitalis within one year of consortship, although three of the consorts contracted their infection from the same index case. Unfortunately, serological data were not obtained routinely from this unvaccinated group of consorts.

Side effects of vaccination

To date, in approximately 900 vaccinations including patients (not computed in this study) who received vaccination to modify recurrent herpetic disease[28a 35] there have been no significant local or general side effects. Immediately after vaccination, most patients complained of a local "stinging sensation" lasting for 30-60 seconds and about 75% of patients had an erythematous reaction with swelling at the vaccination site for 24-72 hours; six vaccinations were followed by a transient "flu-like" syndrome which was at least temporally related to vaccination. Two seronegative and three seropositive consorts were vaccinated in early pregnancy without ill effect to mother or infant.

Discussion

A group of 60 consorts at risk were immunised with antigenoid vaccine Ac NFU_1 (S^-) MRC to prevent

TABLE *Details of vaccination response in 60 sexual consorts of patients with recurrent herpes genitalis*

Vaccine	No in group	No with neutralising antibody response*	Mean k value				No with immunoprecipitating antibody
			Before vaccination		After vaccination		
			HSV type 1	HSV type 2	HSV type 1	HSV type 2	
High-dose							
Seronegative	21	19	0	0	0·37	0·29	9
Low-dose							
Seronegative	13	10	0	0	0·18	0·13	3
Seropositive	26	13	0·49	0·20	0·69	0·39	ND

*Neutralising antibody responses were recorded as positive when there was a significant increase in k value against both type 1 and type 2 herpes virus
ND = not detected

their contracting herpes genitalis. The vaccine contained polypeptides and glycoproteins whose antigenicity had clearly survived the preparative procedure as judged by analyses on polyacrylamide gels and Ouchterlony gel diffusion.

Neutralising antibody responses were obtained in a high proportion of subjects receiving the high dose of vaccine and in about one half of subjects receiving the lower dose. The detection of immunoprecipitating antibody albeit in a lower proportion of post-vaccination sera—which accords with our general experience of the relative prevalence of neutralising and immunoprecipitating antibody in the sera of the general population[28 36]—was encouraging and may provide a simple and convenient initial screening test for seroconversion in large-scale trials of vaccine efficacy.

The rationale of vaccination in seropositive consorts is an open question; however, as we have previously observed that vaccinated mice were significantly better protected than mice who had survived in a live infection[12] and, as in this study, there was a significant neutralising antibody response after vaccination in 13 of 26 subjects with pre-existing antibody (table), it does seem that vaccination offers a quantitatively or qualitatively different immunogenic stimulus. It is possible, for example, that treatment with Nonidet, formaldehyde, or acetone might impart adjuvant-like properties to vaccine polypeptides in terms of their in-vivo stability or immunogenic presentation.

The preventative efficacy of our vaccination programme has been evaluated by comparison with the rate of consort transmission computed prospectively from our experience during prevaccination years. This is not ideal but in some measure confounds the preventative influence of our advice and counselling towards minimisation of the risk of virus transmission between sexual consorts. Vaccinated and unvaccinated groups seemed generally comparable, except that the former were distributed towards a lower socioeconomic status which, if anything, might favour a higher rate of index to consort transmission. Some may have developed a subclinical virus infection but if this did occur, the absence of any clinical recurrences within 18 months is surprising and at worst a welcome modification of the usual clinical outcome.

While the duration of protection is traditionally less with inactivated vaccine preparations, it was encouraging that after immunisation with inactivated vaccines significant levels of protection were obtained for at least 18 months in mice and six months in rabbits and Rhesus monkeys.[35 37] In humans, as there are no unequivocal humoral or cell-mediated immunological correlates of protection against type 2 herpes virus infection, the long-term efficacy of vaccination must await the test of time. We intend to examine the temporal profile of various criteria of the humoral, local secretory, and cell-mediated immune response to varying vaccine dosages and immunisation schedules in the presence and absence of adjuvant and correlate this information with protective efficacy.

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